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DEVELOPMENT OF NOVEL LIQUID PHASE QCRS SENSOR TECHNOLOGY

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Abstract

This paper discusses the development of novel liquid phase quartz crystal resonant sensor technologies for label free, real-time detection of pharmaceutical applications.

New crystal oscillator technologies are introduced alongside crystal mounting and flow cell designs circumventing more traditional approaches to yield greater robustness, reliability and sensitivity. Data for standardised long-term experiments is presented and analysed with respect to reproducibility and stability and comparisons made with surface plasmon resonance.

Keywords: balance, gravimetry, label-free, liquid-phase, quartz crystal resonant sensor

Introduction

Quartz crystal sensor technologies have developed greatly since the initial in-vacuo based work of Sauerbrey [1]. Gas phase applications have been developed for a wide range of analytical purposes including the determination of redox state in conducting polymer films upon the action of organic vapours [2] and water vapour adsorption on gold surfaces [3], with multi-channel instruments being developed to include pattern recognition capabilities [4]. Co-committant advances in the field of electronics allowed the development of oscillator circuitry capable of greater amplitude induction required to achieve crystal oscillation in the highly damped environments of liquid media [5]. A number of different applications have been developed including bacterial binding [6] RNA ligand interaction [7], DNA hybridization [8], with a current assessment of the field provided in an excellent review by Janshoff *et al.* [9]. Although a useful research tool, further development of the apparatus has been partially hampered by a lack of robustness and user friendliness within the current crystal designs and a wide variation in the design of liquid flow cells employed, leading to further problems including differential clamping forces upon crystals and a disparity in the oscillation performance of individual units.

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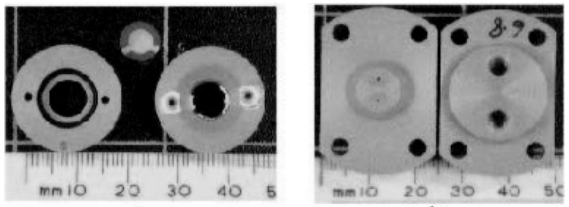
In order to counteract these problems a number of new crystal mount and flow cell designs were investigated, this paper describes the most successful to date and gives details of the real-time performance with model systems whilst comparing a well defined antibody binding assay carried out on both surface plasmon resonance (SPR) [10] and QCRS instruments.

Apparatus

A novel auto gain control (AGC) type oscillator was fabricated, the details of which had been discussed elsewhere [11, 12]. 10 MHz 8.6 mm lapped finish (1 μm), crystals furnished with 5 nm nickel adhesion layers and 50 nm gold working layers were mounted in novel cartridge type crystal holders [13] (Fig. 1a), and further installed into a new type of flow cell (Fig. 1b), (swept volume≈20μL), following a cleaning procedure discussed elsewherwe [7]. Crystal connections were established via a gold pin and socket arrangement. Crystal temperature was maintained within a surrounding aluminium block via custom-built air-cooled peltier devices within the range 4–60±0.1°C. Details of the liquid handling and general apparatus construction have been discussed previously [7]. Automated injections into the flow stream were performed using a Gilson 235 96 well plate autosampler.



a b

Fig. 1 New pressure free cartridge crystal mount (a) and flow cell cap (b)

SPR experiments were performed using a Texas Instruments SPREETA instrument and associated flow cell. Liquid handling was achieved using a Shimadzu LC-9A HPLC pump running at 5 μL min^{-1} connected via a Rheodyne 9125 all PEEK injection valve with 20μL loop. Data acquisition (1 s interval) and analysis was performed using the supplied SPREETA software.

Methods

Solutions of dimethyl sulfoxide in water were used as liquid phase standards due to the large density/concentration characteristics of the mixtures and its low affinity for the crystal surface. Initially all wells of a 96 well plate were filled with a DMSO solution at 10% *v*/*v*. Samples were introduced to a water stream flowing over the crystal, (5 μL min^{-1}), at 10 min intervals with frequency and AGC controller output voltage being recorded at one second intervals. Samples were injected over a sixteen h period. These

experiments were followed by similar runs where triplicate injections of DMSO were made with concentrations of 1, 2, 5 and 10% *v*/*v*. Following repeated DMSO injections a single injection of NeutrAvidin™ (1 mg mL^{-1}), was made in order to assess the long-term stability and response of the crystals when compared to unused units.

Comparison of the sensitivity and stability of the new QCRS instrument and crystal flow cell was made with the established technique of SPR. Cleaned gold surfaces were employed in both instruments. Following the establishment of filtered (0.2 μm), phosphate buffer baselines (pH 7.2), within both instruments, an aliquot of human fibrinogen (1 mg mL^{-1}), was passed through each system, being followed by after 300 s by an injection of an anti-human fibrinogen antibody.

Results and discussion

Sequential injections of 10% *v*/*v* DMSO over sixteen h led to ninety-six individual frequency and AGC peaks with little sign of tailing, of 350±SD1.2Hz, (*N*=10) and 112±SD 2 mV (*N*=10), respectively, with baseline noise below 0.5 Hz and the equivalent of less than 1 Hz drift per sample. Fitting of individual frequency peaks to a Gaussian curve fitting model using Graphpad Prism™ led to fits with r^2=0.958 (±0.02, *N*=6), and through cell flow times of 325 s (compared to theoretical value of 240 s for a 20 μL injection at 5 μL min^{-1}), suggesting strongly that hydrodynamic performance within the flow cell is non turbulent, that cell swept volume was closely matched to that of the injection loop but that projected flow rates were over rated by approximately 30% between pump and flow cell. Figure 2 shows the effect of 10% *v*/*v* DMSO injections upon the system and also the effect of reduction in DMSO concentration. A strong concentration dependence on both frequency and AGC was observed, which in the case of DMSO if plotted led to a linear relationship within this range for both parameters (r^2=0.9994 frequency, r^2=0.9924 AGC).

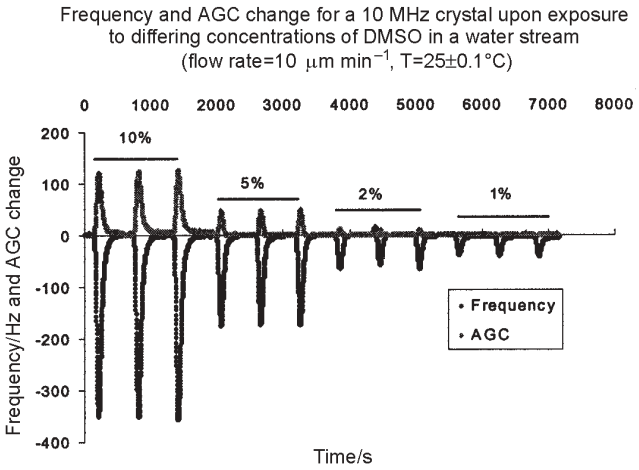


Fig. 2 Repeatability and concentration response data using model DMSO in water standards

Injection of an aliquot of NeutraAvidin (1 mg mL^{-1}), to a crystal following 96 sequential injections of 10% *v/v* DMSO with accordant transient frequency and AGC shifts, led to residual frequency shifts of –200 Hz, but no measurable AGC change away from the pre-existing buffer baseline. The measured frequency change was very close to those measured in similar experiments [14] without previous DMSO exposure or sixteen h runtime (–180±SD 22, *N*=8). This shows that crystals mounted in the new holder are unaffected by both long periods of oscillation and exposure to the DMSO. Further this supports the notion that frequency and AGC are uncoupled parameters and capable of individually being affected by both solution properties and binding from solution.

Analysis of both SPR (Fig. 3a) and QCRS (Fig. 3b), data for the binding of both human fibrinogen and its specific antibody shows clearly that whilst SPR is clearly capable of clear and precise measurement of the applied analytes, QCRS techniques are more than capable of matching the performance of the SPR employed and actually yield a greater change of frequency per unit volume (600 Hz/0.1 μg protein, 1200 Hz/0.1 μg antibody) compared to SPR angle (500 mda/0.1 μg protein, /350 mda/0.1 μg antibody) and in this case, when comparing binding curve structure that loop volume and flow rate appear to be better matched to the QCRS flow cell than to that of the SPR instrument.

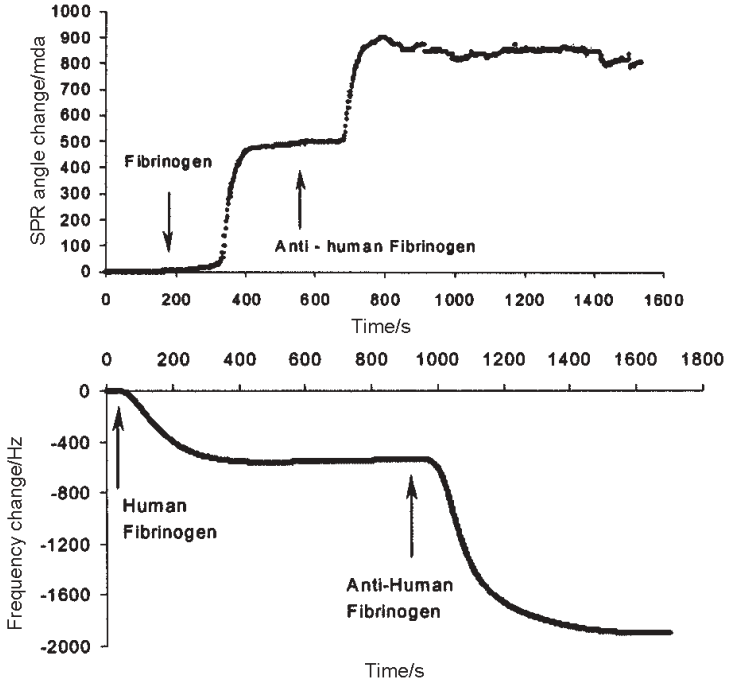


Fig. 3 a – SPR and b – QCRS profiles for the non-specific attachment of human fibrinogen to a gold sensor surface followed by an anti-human fibrinogen antibody

Conclusions

Novel quartz crystal oscillators and crystal mountings have been developed as part of an instrumental base that has been proven stable and robust over extended periods with model systems. The new crystal mounting system would potentially greatly improve the robustness and reproducibility of the transducer elements and hence the overall accessibility of the technique. Further, the technique has been shown capable of improving upon current SPR instrument performance with a useful protein – antibody assay. Fusion of oscillator technologies with plate autosampling capabilities allows the theoretical throughput of a single channel instrument to be in excess of 150 samples per day.

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